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Effect of external and internal factors on the germination of fungous spores*

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(WITH TWO TEXT FIGURES)

The greater part of the work which has been done on the germination of fungous spores has been incidental to studies of the life histories of fungi. References to spore germination are in most cases isolated and few attempts have been made to draw any general conclusions from the results obtained. Since all fungous diseases must begin with infection and since the germination of the spore is the forerunner of infection, further knowledge of the conditions which favor or inhibit spore germination is of great practical importance. In this paper are described the results of the writer's study of the effect of various factors on the germination of fungous spores.

It is becoming increasingly common for field studies of the efficiency of fungicides to be preceded by laboratory tests of the toxicity of fungicides to fungi. If fungous spores are to be germinated both in the presence and absence of fungicides, it is important that all other conditions be identical and at or near the optimum. It is therefore necessary that optimum conditions for the germination of the spores be known before the laboratory tests of fungicides are made.

Frequent references are made in the literature to the effect of prevailing climatic conditions on the outbreak or severity of

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epiphytotics of fungous origin. Field experiments and observations on the effect of weather on diseases of plants are affected by a very complex set of factors. Conclusions cannot safely be drawn until the various factors have been separately studied and such study is more easily effected in the laboratory than in the field.

Factors influencing the germination of fungous spores may be divided into two groups, internal and external. The internal factors are maturity of the spore, longevity of the spore, animation, and a poorly understood factor which may be called vitality of the spore. The external factors are oxygen, presence of moisture either in the form of water vapor or water of condensation (rain or dew), temperature, light, nutrient substances, toxic substances, and duration of the time period during which one or more than one of the factors acting jointly exert their influence on the spore of the fungus.

EXPERIMENTAL METHODS

The germination of the following fungous spores was studied: conidia of *Venturia inaequalis* (Cke.) Wint., conidia of *Sclerotinia fructigena* (Pers.) Schrt., spores of *Alternaria Solani* (E. & M.) Jones & Grout., spores of *Botrytis cinerea* Pers., spores of *Rhizopus nigricans* Ehr., aeciospores and urediniospores of *Cronartium ribicola* F. de Wal., aeciosopres of *Gymnosporangium clavipes* C. & P., and teliospores of *Puccinia Malvacearum* Mont. The spores of the fungi were obtained fresh from the living host in all cases except *Alternaria Solani*. Spores of parasitic fungi to be used for germination tests were found to be more dependable when obtained from this source than when obtained from fungi growing on artificial media. A spore obtained from the living host is presumably possessed of its full natural vigor. A spore obtained from a fungus growing on artificial media may have been subjected to a debilitating influence, or may, on the other hand, have become unnaturally resistant to adverse conditions. In this connection it should be noted that Young and Cooper (1) found that when *Glomerella rufomaculans* was grown on agar eight to fifteen days, the spores were more resistant to the toxic action of fungicides than when the fungus had been grown on agar only four to eight days. If fresh spores from the living host are not obtainable and spores from artificial culture must be used, it is the experience of the writer that all spores

the germination of which is to be studied should be of the same age. The germination of spores from a second culture transferred from the first should not be compared with the germination of spores from the first culture.

Distilled water was the medium in which the spores were placed to germinate. As is subsequently described more in detail, it was soon evident that the distilled water contained an insufficient amount of air for spore germination. Consequently, the distilled water was in all cases aerated before being used. The aeration was accomplished by drawing air through the water for about thirty minutes by means of an aspirator and an Allihn gas washing bottle.

On culture plate benches in moist chambers were placed glass slides and on them drops of water either containing spores in suspension or the spores were shaken off into the drops. It is important that all drops of water be approximately equal in size especially when the toxic action of fungicides is to be tested. Clark (**2**) studied spore fermination in hanging cultures. Duggar (**3**) used that method also and a modification of it, placing the cells in small Petri dishes. Mains (**4**) germinated fungous spores in hanging drops on the cover of a Petri dish.

When the cardinal temperatures for the germination of the spores being studied was unknown, the first step consisted in the determination of the optimum temperature for germination, and in all later tests the spores were germinated at their optimum temperature. Constant temperatures were secured by the use of a DeKhotinsky electric oven and a Hearson incubator.

The next point determined was the minimum length of time in which all the viable spores of each fungus will germi ıate at the optimum temperature. At least six hours more than this minimum time period was allowed to elapse before removing the slides from the moist chambers a ıd determining the relative number of spores germinated. If slightly less than sufficient time is allowed a narrower range of optimum temperatures will be found than if more time is allowed, resulting, when the results are plotted, in a temperature-germination curve more acute than flat at its summit. If too long a time passes before the germinating spores are counted, the germ tubes will have grown and branched so as to make counting difficult and uncertain. *All* data are based on relative number of spores germinating.

The spores were counted by the use of a micrometer disc ruled in squares and the help of a tallying register. The micrometer disc ruled in squares is placed in the ocular, the slide bearing the drops of water containing spores is placed on the stage of the microscope and moved by means of a mechanical stage until the outer line on the micrometer disc appears as a tangent to the circumference of the drop. The germinating spores appearing between the first two lines of the micrometer disc are counted, and then the ungerminated spores in this area are counted. When one field as limited by the lines on the micrometer disc is counted, the slide is moved enough to bring a new field into view for counting. This is continued until a total of fifteen hundred to two thousand spores, both germinated and ungerminated, have been counted. This necessitates the examination of several or many drops. From the count obtained, the relative number of spores germinating is figured, germination elsewhere being raised proportionately. This accuracy of counting is most necessary when the relation between temperature and spore germination is being determined. It is not as necessary in the case of toxicity tests with fungicides except when the differences in toxicity are small.

RELATION OF THE VIABILITY OF THE SPORE TO ITS AGE

Maturity.—Until the spore has attained a certain age or degree of maturity, it cannot germinate. Even before a spore is really mature, it can germinate if other conditions are near enough to the optimum. For example, the range between the minimum and maximum temperature is considerably greater for the germination of a mature urediniospore of *Puccinia Antirrhini* than for the germination of an immature urediniospore of this fungus, collected the first day it breaks through the host surface. The immature spore may be prevented from germinating by the action of fungicides not of sufficient concentration to prevent the germination of the mature spore. A solution containing 0.257 per cent copper sulphate prevents the germination of mature spores of *Uromyces caryophyllinus.* A solution containing only half as much copper sulphate prevents the germination of immature spores of this fungus. It should be understood that mature and immature spores may not differ morphologically. Spores here referred to as immature are so called because when collected young they did not germinate,

although older spores from the same fungus did germinate. All conclusions as to germination should be based upon the behavior of mature spores.

It is possible by exercising care in obtaining spores to separate the mature from the immature to some extent at least. When a fungus has matured its spores, they are usually freed in such a way that they may be disseminated by the movement of air or water. So far as possible, the natural method should be duplicated in obtaining spores for experimental purposes. When a scab lesion on the fruit or leaf of the apple is washed, it is probable that only mature conidia and perhaps a few dead conidia of *Venturia inaequalis* are detached. But when a similar lesion on the fruit or leaf of the apple is brushed or scraped, it is probable that many immature conidia are also detached. In the case of all except the fungi having very short-lived spores the immature spores on a fresh lesion greatly outnumbered the dead spores. When the writer obtained conidia of *Venturia inaequalis* by brushing apple scab lesions the relative number of conidia which were capable of germinating was only twenty-five as compared with the relative number of one hundred germinating when the conidia were obtained by washing lesions with a stream of water from a pipette. When fungous spores are to be obtained for germination studies they should be freed from the host as gently as possible and in as nearly as possible the same way in which they would be removed in nature. In this connection it is interesting to note that Jones (**5**) found that the ascospores of *Pseudopeziza Trifolii* and *P. Medicaginis* germinate readily when they are discharged naturally but not when they are crushed out of the ascus.

Some attention has been given to the later maturing of spores which were detached from the fungus while still immature. Schaffnit (**6**) is of the opinion that unless fungous spores are internally mature before they are detached from the fungus, they never gain power to germinate. But the results of Melhus and Durrell (**7**) indicate that some urediniospores can mature after being detached from the fungus.

With the exception of the spores of *Botrytis cinerea*, all the fungous spores studied by the writer were apparently killed if detached when immature.

It is possible in some cases to distingiush between mature and immature spores by their relative position in the fruiting parts

of the fungus. The only spores of *Sphaerotheca mors-uvae* which Foreman (**8**) induced to germinate were the large spores found at the tips of the chains of spores. Weimer (**9**) observed that the teliospores of *Gymnosporangium Juniperi-virginianae* which are situated at the apex of the spore horn germinate best and he attributed this to their maturing earlier.

As the season advanced in the autumn the writer found it increasingly difficult to secure good germination of the conidia of *Venturia inaequalis* even when conidia were obtained from fresh lesions. This is not attributed to the presence of a greater number of spores too old to germinate, for they would probably have been washed away earlier. It is believed that a larger number of immature conidia of this fungus were present in the autumn. It is not unlikely that the process of maturing of the spore is slower when the temperature is lower.

Very little work has been done on the relation of the viability of fungous spores to the conditions under which the host plant grew and in which the fungus developed. If certain conditions may retard the maturing of the spores, it is quite possible that other conditions during their development may entirely prevent their ever germinating. On the other hand, certain conditions during development of the spore may result in an extremely vigorous spore. Spores may be mistakenly designated as mature and immature, on the basis of their behavior when placed under optimum conditions for germination, when they should properly be designated as spores of the same age, some vigorous and others without vigor.

The inability to germinate any fungous spore under all possible combinations of conditions may be temporarily explained by the supposition that the spores are either dead, non-functioning or in a resting condition. There are perhaps many resting spores not now recognized as such. Reed and Crabill (**10**) have advanced the theory that a rest period is necessary for the aeciospores of *Gymnosporangium Juniperi-virginianae* and that these spores do not germinate until the spring following their dispersal.

Longevity.—For all spores there is a maximum time limit, variable with the environmental conditions, beyond which the spore cannot germinate. The spore may be alive and too old to germinate or it may be dead. A live spore too old to germinate under the range of conditions within which it formerly could germinate is, according to the observation of the writer, able

to germinate when all conditions approach the optimum. There comes a time when even this is not stimulative and the spore is then dead so far as we can tell. Neglect of the age factor may render any study of spore germination undependable.

In studying the relation of the age of spores to their viability, points to be considered are the date of collection, the conditions in which the spores are placed after collecting, and the length of time spores remain viable after their collection. Aeciospores of *Cronartium ribicola* were found to germinate with diminishing vigor as the season advanced. The aeciospores of this fungus were collected on May 25th, June 4th and June 12th from the same sorus. On the first date, the relative number of spores germinating was one hundred, on the second date forty-nine and on the third date twenty-nine. Observations with this and other fungi indicate that although there is at first a sharp falling-off in viability, it is retained for a comparatively long time and only gradually lost. In any spore germination studies which involve comparisons, spores should be used which are known to be of the same age.

Among the factors bearing upon the retention of viability after the collection of spores are moisture, temperature, and freedom from or connection with the parent. Detachment from the parent was not found to have any effect on the length of life of the aeciospores of *Cronartium ribicola*, viability decreasing the same in both cases. When collected, the relative number of these spores germinating was one hundred, after having been collected thirty days the germination fell to seventy-three, after fifty days to five, and after fifty-five days no spores germinated.

Freshly collected aeciospores of *Cronartium ribicola* were stored indoors at temperatures of 7° C., 15° C., and 23° C. These various temperatures had no effect upon the longevity of the spores. But aeciospores stored in moist air retained their viability longer than those stored in dryer air. At least in the case of this fungus moisture is of more importance than temperature in its effect on the length of life of the spore. This effect of moisture is to be expected; for all spores lose water in dry and moist air, and if other conditions are equal they should live longer in moist air than in dry air. These results agree with those of Anderson (**11**) who found that in a humid atmosphere

the spores of *Cylindrocladium scoparium* live for several weeks but that in a dry atmosphere they die in fifteen days.

Under no conditions did aeciospores of *Cronartium ribicola* germinate when more than eight weeks old. A few cases are recorded in the literature of aeciospores of this fungus living longer, but it is probable that in such cases germination is reduced to a trace and that even that small germination occurs only when all environmental conditions are very close to the optimum for a time.

The conidia of *Venturia inaequalis* produced on the leaves are somewhat shorter lived than those produced on the fruit. According to Aderhold (**12**) the conidia of this fungus retain their viability not more than eight weeks. The writer found none of the conidia of this fungus to live more than six weeks on the fruit and not more than four weeks on the leaf. With the aging of the leaves in the fall, the life of the conidia becomes even shorter. Difficulty was experienced in germinating conidia from fruit in storage. The scab lesions, however, were contaminated with *Cephalothecium roseum*, the presence of the conidia of which in the drops of water may bave been partly responsible for the failure of the conidia of *Venturia inaequalis* to germinate. As will be described later, the presence in a drop of water of the spores of several fungi prevents or retards the germination of all the spores as compared with germination in a drop of water containing only the spores of one fungus.

One of the factors bearing upon the retention of viability by spores after their collection is their freedom from or connection with the fungus or each other. Anderson and Rankin (**13**) found that ascospores of *Entothia parasitica* which remained in the perithecia in the bark germinated after being stored dry for one year. But when ascospores were removed and separated for only five months they lost the power to germinate. According to Burrill (**14**) the spores of *Glomerella rufomaculans* remain viable for a long time when their soluble protective coating in which they are imbedded is present. But if it is washed away, the spores soon lose the power of germination. Anderson and Rankin (**13**) found that if the pycnospores of *Entothia parasitica* are stored in a dry place they live at least one year. But if the pycnospore horns are placed in water and the water allowed to evaporate so as to leave the spores separated, they do not retain their viability more than one month.

It is possible that in most cases the mucilaginous covering in which many spores are imbedded serves to prolong the life of the spore. For this reason, when studying the germination of such spores, it is advisable that only those still adhering together in their protective coatings be used.

Exposure to light shortens the life of some spores, as shown by the results of Hoerner (**15**) with the urediniospores of *Puccinia coronata*.

Some attention has been given to the longevity of the spores of the Uredinales. The sporidia are the shortest-lived spores of this group of fungi. According to Reed and Craybill (**10**) the sporidia of *Gymnosporangium Juniperi-virginianae* do not live more than six days in dry air. Spaulding (**16**) reports that the sporidia of *Cronartium ribicola* live less than ten minutes at room temperature with the humidity at ninety. Duff (**17**) found that two weeks after the collection of the urediniospores of *Cronartium ribicola* their germination was so reduced as to become practically negligible. The life of a teliospore measured from the time of its formation to its death may be long, but the life of a teliospore if measured from the close of its normal resting period until its death is not long; it is apparently as short in some cases as the life of many non-resting spores. Melhus, Durrell, and Kirby (**18**) report that the teliospores of *Puccinia graminis*, which mature in April, cannot germinate after the end of June following.

The experience of the writer is to the effect that in most cases aeciospores are longer lived than urediniospores. The average life of urediniospores is between thirty and sixty days. The average life of the aeciospore is about 50 per cent greater. The several spore forms of the Uredinales behave alike in this, that as they grow older their range between maximum and minimum conditions for germination becomes narrower.

EXTERNAL FACTORS

Temperature relation.—One of the most important external factors bearing upon the germination of fungous spores is the temperature relation. In order to draw from the scattered data in the literature some more general conclusions than we now have as to the cardinal temperatures for the germination of fungous spores, TABLE I has been prepared. In it are given the cardinal

temperatures and the range of temperature for germination of thirty fungous spores.

If the optimum temperature for the germination of the spores of *Plasmodiophora Brassicae*, which Chupp (**19**) found to be 27°–30° C., is typical for the Myxomycetes, then the temperature requirements for the germination of the spores of the slime moulds must be higher than those of fungi.

A study of TABLE I shows for the germination of the spores of several groups of fungi the following average cardinal temperatures and average range between minimum and maximum temperatures. For the germination of the Phycomycetes the average cardinal temperatures are 1.2° C., 18° C., and 26.1° C., and the range is 25.0 degrees Centigrade. For the germination of the aeciospores of the Uredinales, the average cardinal temperatures are 6.5° C., 13.0° C. and 22.0° C. and the average range is 15.5 degrees Centigrade. The average cardinal temperatures for the germination of the urediniospores are 5° C., 16.2° C., and 28.9° C., and the range through which these spores can germinate is 23.9 degrees Centigrade. In the case of teliospores the cardinal temperatures for germination are 7.4° C, 19.9° C., and 28.1° C., and the range for germination is 20.7 degrees Centigrade.

Temperature limits are narrower for the production of sporidia by germinating teliospores than for the production of germ tubes only. This is illustrated by the results obtained by the writer with the teliospores of *Puccinia Malvacearum*. At temperatures as low as 5° C. promycelia are produced but no sporidia. Above 23° C. promycelia are produced but sporidia are rare.

These results agree with those of Dietel (**29**) who found that above 23° C. the teliospores of *Puccinia graminis* produce no sporidia but only germ tubes. Reed and Craybill (**10**) noticed that the teliospores of *Gymnosporangium Juniperi-virginianae* do not germinate when the temperature rises to 24° C. But above that temperature they sometimes develop promycelia.

When the Fungi Imperfecti and the one representative of the imperfect stage of the Ascomycetes named in TABLE I are considered collectively, it is seen that for the germination of these spores the cardinal temperatures are 7.3° C., 23.1° C., and 39.6° C. Between the minimum and maximum temperature limits for germination there is an average range of 32.3 degrees Centigrade.

TABLE I

Cardinal temperatures and range of temperature for the germination of the spores of representative fungi.

Authority*	Fungus	Cardinal Temperatures			Range
		Min.	Opt.	Max.	
18	*Plasmodiophora Brassicae*	—	27°–30° C.	—	—
20	*Plasmopora viticola*, conidia	—	25°–30° C.	—	—
21	*P. viticola*, conidia	—	25°–35° C.	—	—
22	*Cystopus candidus*, conidia	0° C.	10° C.	25° C.	25
23	*Phytophthora infestans*, conidia	2°–3° C.	12°–13° C.	24°–25° C.	23
24	*Peronospora parasitica*, conidia	—	8°–12° C.	29° C.	—
25	*Gymnosporangium clavipes*, aeciospores	8° C.	14° C.	25° C.	17
25	*Cronartium ribicola*, aeciospores	5° C.	12° C.	19° C.	14
4	*Puccinia Phlei-pratensis*, urediniospores	—	18° C.	30° C.	—
4	*P. coronata*, urediniospores	—	18° C.	30° C.	—
26	*P. coronata*, urediniospores	7° C.	—	30° C.	23
7	*P. coronata*, urediniospores	1° C.	17°–22° C.	35° C.	34
26	*P. rubigo-vera*, urediniospores	2° C.	—	31° C.	29
26	*P. graminis*, urediniospores	2° C.	—	31° C.	29
27	*P. dispersa*, urediniospores	10°–12° C.	18°–20° C.	25°–27° C.	17
25	*P. Antirrhini*, urediniospores	5° C.	10° C.	20° C.	15
28	*P. Sorghi*, urediniospores	4° C.	14° C.	25° C.	17
25	*Cronartium ribicola*, urediniospores	8° C.	14° C.	25° C.	17
25	*Uromyces caryophyllinus*, urediniospores	4° C.	14° C.	29° C.	25
4	*Uromyces Trifolii*, urediniospores	—	16° C.	34° C.	—
10	*Gymnosporangium Juniperi-virginianae*, teliospores	11° C.	15° C.	29° C.	18
9	*G. Juniperi-virginianae*, teliospores	7° C.	23°–24° C.	29° C.	22
29	*Puccinia graminis*, teliospores	9° C.	22° C.	23° C.	14
18	*P. graminis*, teliospores	5° C.	20° C.	25° C.	20
29	*P. Larici*, teliospores	6° C.	—	—	—
25	*P. Malvacearum*, teliospores	3° C.	14° C.	30° C.	27
18	*P. graminis*, basidiospores	—	15°–20° C.	—	—
30	*Colletotrichum lagenarium*	7° C.	22°–27° C.	—	—
31	*Melanconium* sp.	—	23°–27° C.	—	—
32	*Phyllosticta Antirrhini*	—	25° C.	—	—
33	*P. Antirrhini*	18° C.	—	47° C.	29
34	*Alternaria Solani*	1°–3° C.	26°–28° C.	37°–45° C.	44
35	*Septoria* sp.	—	24°–28° C.	34° C.	—

* Numbers refer to the literature cited.

For these several groups of fungi the minimum temperatures for spore germination occur between 1° and 7.4° C. The optimum temperatures are all between 13° and 23.1° C. The maximum temperatures for the germination of the spores occur between 22° and 39.6° C. The Phycomycetes have the lowest minimum temperatures, followed in order by the urediniospores, the aeciospores and the teliospores of the Uredinales. Teliospores and the spores of the Fungi Imperfecti have the highest, and about the same minimum temperature for germination.

Of these five groups of fungous spores, aeciospores have the lowest optimum temperature for germination followed in turn

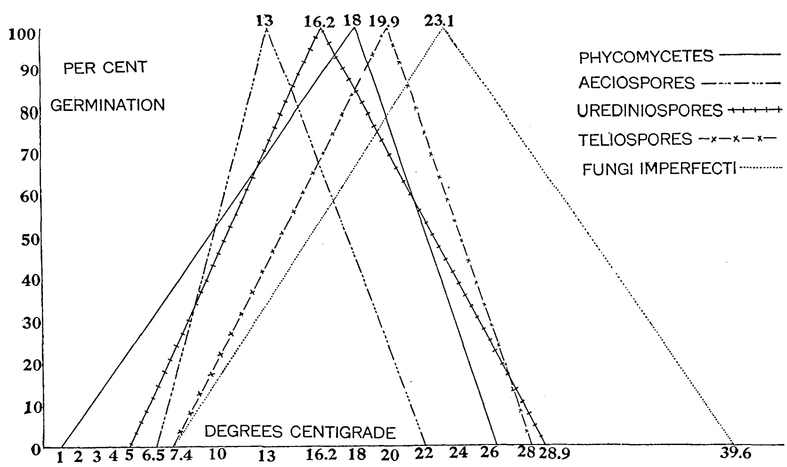

FIG. 1. Curves showing the cardinal temperatures for the germination of the spores of representative fungi.

by urediniospores, conidia of Phycomycetes, teliospores, and then by the spores of the Fungi Imperfecti, which seem to have the highest optimum temperature for germination.

Aeciospores have the lowest maximum temperature for germination, and the spores of the imperfect fungi have the highest. About midway between these two come urediniospores. Their maximum temperature for germination is higher than that of the conidia of the Phycomycetes.

The spores of the Fungi Imperfecti can, it would appear, germinate through the greatest range of temperature. As compared with this, the range of the Phycomycetes is 77 per cent

as great, that of the urediniospores is 74 per cent as great, that of the teliospores is 64 per cent as great, and the range of the aeciospores is only 48 per cent.

FIG. 1 shows the ranges and the cardinal temperatures for these groups, and their relative positions.

The results obtained by the writer and others indicate that any departure from optimal conditions tends to narrow the temperature limits within which fungous spores will germinate. The literature contains a very few references to the relation which

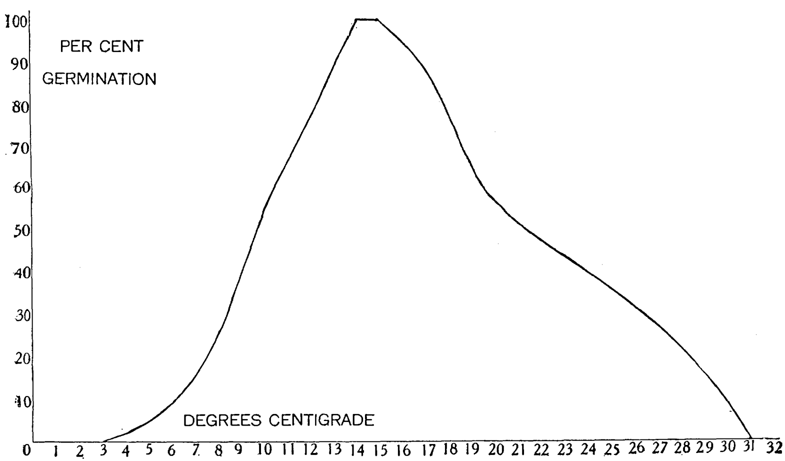

FIG. 2. Curve showing temperature limits for the germination of the conidia of *Venturia inaequalis*.

exists between the temperatures required for the process of spore germination and the other environmental conditions prevailing during that period. Gardner (**30**) found that the minimum temperature for the germination of the spores of *Colletotrichum lagenarium* is 7° C. when the spores are in exposed drops of water, but it is 14° C. when the spores are in hanging drops of water. When these spores are in exposed drops of prune decoction, the minimum temperature for their germination is 4° C. It would appear that the presence of nutrient substance and the availability of sufficient oxygen both tend to lower the minimum temperature.

The work of the writer included the determination of the cardinal temperatures for the germination of the conidia of *Venturia inaequalis*, the spores of *Botrytis cinerea*, and those of

Rhizopus nigricans. Cardinal temperatures for the germination of the other spores used had been previously determined (Doran, **25**).

The results obtained with the conidia of *Venturia inaequalis* are shown in TABLE II and by the curve in FIG. 2. For the germination of the conidia of *Venturia inaequalis*, the minimum temperature is 3° C., the optimum temperature is 14°–15° C., and the maximum temperature is 31° C. The conidia can germinate through a range of twenty-eight degrees.

This is an unusually long range. Since the causal fungus of apple scab is not especially exacting as regards temperature for the germination of the conidia the indications are that the distribution or severity of this disease, following the primary infection by the ascospores is dependent more upon rainfall than upon temperature. The conidia of *Venturia inaequalis* are disseminated most freely during June, July and August. As shown in FIG. 2, warm nights do not prevent their germination.

A study of the temperature-germination curve in FIG. 2 shows that the falling off in germination of the conidia is more rapid from the optimum to the minimum temperature than from the optimum to the maximum temperature. Over 40 per cent of the number of spores germinating at the optimum (14°–15° C.) germinate at 9° C. and 24° C., respectively.

TABLE II

Effect of temperature on the germination of the conidia of *Venturia inaequalis*

Relative germination at different temperatures measured in degrees Centigrade

	2°	3°	5°	8°	10°	12°	14°	15°	18°	20°	24°	28°	30°	31°	32°
	0	1	2	20	52	76	100	100	90	61	42	26	14	1	0
	0	0	4	18	60	80	100	100	68	58	46	24	10	2	0
	0	1	1	23	55	69	100	100	70	55	38	20	12	0	0
	0	1	5	25	58	81	100	100	81	52	40	19	8	3	0
Mean	0	0.75	3	21.5	56.2	76.5	100	100	77.2	56.5	41.5	22.2	11	2	0

In these studies of the relation of spore germination to temperature, as to all other external conditions, the relative number of spores germinating was taken as an indicator of the reaction

of temperature. Johnson (**26**) and Melhus and Durrell (**7**) preferred to use the growth or length of the germ tubes as an indicator of the temperature reaction. To test the relative merits of these indicators, both were used by the writer in the case of germinating conidia of *Venturia inaequalis*. When the optimum temperature for germination was based upon length of germ tube rather than upon number of spores germinating the optimum was less sharply defined. The writer believes that to consider length of germ tube rather than number of spores germinating involves growth rather than germination and so should be studied separately, for there is no reason to believe that the optimum temperature for the growth of a fungus is the same as the optimum temperatures for spore germination. Melhus (**22**) found the optimum temperature for the germination of the conidia of *Phytophthora infestans* to be 12°–13° C. Jones, Giddings and Lutman (**30**) found that the optimum temperature for the growth of this fungus is between 16° and 19° C. The cardinal temperatures for the germination of the conidia of *Botrytis cinerea* were found to be minimum 7° C. to 17° C., and maximum 26° C. For the germination of the spores of *Rhizopus nigricans*, the minimum temperature was found to be 10° C. and the optimum 19° C. to 20° C. All later studies of the germination of *Botrytis cinerea* were made at 15° C. and of the germination of *Rhizopus nigricans* at 19° C.

Time relation.—The length of time required for the process of spore germination is in itself an important factor. If before the elapse of the necessary time period the environmental conditions depart from the optimum, the germination process is impeded or stopped and the spore may even be killed. In all laboratory studies of spore germination, if conditions in nature are to be duplicated, no more time need be allowed for germination than that in which nearly optimal conditions are likely to continue in nature. Probably most fungous spores begin and complete germination between darkness and daybreak. One of the principal natural protections of plants from pathogenic fungi is the failure of environmental conditions to remain between minimum and maximum limits long enough for spore germination. Infection does not occur if during the protrusion and growth of the germ tube the necessary precipitated moisture evaporates or the air falls below a certain degree of humidity or the temperature passes beyond minimum or maximum limits.

According to the observation of the writer, extremes of temperature at this time, if not too long continued, have no more serious effect than to temporarily stop the growth of the germ tube. This is resumed when the temperature again approaches the optimum. But when the humidity of the air falls, the results to the spore are more serious. In the case of all spores worked with, and especially with conidia of *Venturia inaequalis*, it was found that a germinating spore can live but a short time in a dry environment, which to an ungerminated spore would be in no way detrimental. A return to optimal conditions proved such spores to be not merely inhibited but dead.

Wallace (**37**) considered the length of time apple trees remain wet so as to allow spore germination an important factor in determining whether or not a certain rain would permit infection by *Venturia inaequalis*. He found that the ascospores of this fungus can germinate in four hours but he believed that apple trees must remain wet eight or ten hours to be abundantly infected. The writer will here comment that the further the temperature is from the optimum for germination, the longer must the surface of any plant remain wet in order for the spores on it to germinate and infect the host.

Duggar (**3**) found that after drying the germinating spores of *Botrytis* would grow no more. But the germinating spores of *Aspergillus* showed new growth after being dried several days. Duggar's results with the parasitic form mentioned are in agreement with the results secured by the writer, who worked only with parasitic forms.

The relation of environmental factors to the time required for spore germination has occasionally been noted. The amount of moisture present may affect the length of time in which the spore germinates. Taubenhaus (**38**) reports that the spores of *Glomerella rufomaculans* from the sweet pea germinate in from six to twenty-four hours, depending on whether there is much or little moisture present in the atmosphere.

The writer has noticed that spores germinating in moist air require a relatively longer time for the process than do the same spores germinating in or on precipitated moistqre.

Anderson (**11**) observed that at 25° C., which is within optimal limits for the germination of the spores of *Cylindrocladium scoparium*, the spores of this fungus begin to germinate in two to three hours, but at lower temperatures a longer time is neces-

sary for their germination, five hours at 12° C. and twenty-four hours at 8° C. Tisdale (**39**) found that at 4° C., which is their minimum temperature for germination, the spores of *Didymellina iridis* germinate in twenty-one hours, but at their optimum temperature of 20° C. to 26° C. these spores germinate in two and one half hours, and at their maximum temperature which is 30° C. their germination requires eleven hours.

The presence of a nutrient solution may hasten the germination of some spores. Gardner (**30**) found that the spores of *Colletotrichum lagenarium* germinate in five hours in agar but require ten to twenty-four hours for their germination in distilled water at the same temperature.

It appears that immature spores and aged spores may germinate more slowly than mature spores. Melhus, Durrell, and Kirby (**18**) found that both before and after the teliospores of *Puccinia graminis* have completed their rest period, the time required for their germination is longer than that required just as they end their rest period in the spring.

The results mentioned above indicate that all fungous spores germinate most rapidly when all environmental conditions are nearest to the optimum.

TABLE III shows the time necessary for the germination of twenty-three representative fungous spores. After a study of this table it is possible to draw certain conclusions as follows: The average time required for the germination of fungous spores is about twelve hours; for the germination (indirect) of the conidia of the Phycomycetes it is four hours; for the ascospores of the Ascomycetes it is thirteen hours; for the germination of the chlamydospores of the Ustilaginales it is twenty-one hours; for the germination of the aeciospores of the Uredinales it is ten hours; for the germinations of the urediniospores of the Uredinales it is five hours; for the germination of the teliospores of the Uredinales it is three hours; and the average time required for the germination of the spores of the Fungi Imperfecti is seventeen hours.

The writer has found that the length of time necessary for the germination of the fungous spores named in TABLE IV is: aeciospores of *Cronartium ribicola* twelve hours; urediniospores of *Cronartium ribicola* five and one-half hours; for the production of basidia by the teliospores of *Puccinia Malvacearum* two hours; one hour more, a total of three hours are necessary for the

production of sporidia by the teliospores of this fungus; aeciospores of *Gymnosporangium clavipes* require four hours; conidia of *Venturia inaequalis* about twenty-four hours; and conidia of *Sclerotinia fructigena* five hours. These tests were all made at

TABLE III

Length of time required for the germination of the spores of representative fungi

Authority*	Fungus	Hours necessary for germination
23	*Phytophthora infestans*, conidia	1–2
22	*Cystopus candidus*, conidia	2–10
40	*Plasmopara viticola*, conidia	3–12
37	*Venturia inaequalis*, ascospores	4
41	*Guignardia Bidwellii*, ascospores	36
13	*Endothia parasitica*, ascospores	6–12
39	*Didymellina Iridis*, pycnospores	18–36
	ascospores	6
5	*Pseudopeziza Trifolii*, ascospores	12
42	*Diplocarpon Rosae*, conidia	24
38	*Glomerella rufomaculans*, conidia	6–24
43	*Tilletia foetens*, chlamydospores	48
43	*Ustilago Hordei*, chlamydospores	6.5
43	*Ustilago Tritici*, chlamydospores	14–15
25	*Cronartium ribicola*, aeciospores	8–10
25	*Puccinia Antirrhini*, urediniospores	5–8
44	*P. Phlei-pratensis*, urediniospores	3
35	*P. Malvacearum*, teliospores	3
9	*Gymnosporangium Juniperi-virginianae*, teliospores	3–4
30	*Colletotrichum lagenarium*	10–24
45	*C. Schizanthi*	16–24
32	*Phyllosticta Antirrhini*	16–30
33	*P. Antirrhini*	18–24
11	*Cylindrocladium scoparium*	2–3.5
46	*Septoria Gladioli*	18

* Numbers refer to the literature cited.

the respective optimum temperatures for germination. Other tests were made at temperatures approaching the minimum and maximum temperatures for the germination of the spores of these fungi. Under these conditions, in every case, a longer time was required for germination than at optimum temperatures.

Oxygen relation.—It was observed that spores in the interior of a drop of distilled water never germinated as well as those on

the surface. The conidia of *Venturia inaequalis* germinated only when they were on or near the surface of the water. Teliospores of *Puccinia Malvacearum* did not produce sporidia when deeply submerged in the drop. But when distilled water was used, which had previously been aerated, equally good germination was secured whether or not the spores were submerged in the drops.

DeBary (**47**) described the behavior of spores in water between a cover glass and a glass slide. In this case, the spores

TABLE IV

Time period necessary for the germination of representative fungous spores under optimum conditions

Fungus and spores	No. of hours	Spores germinating (relative numbers)
Cronartium ribicola, Aeciospores	10 12	0 100
Cronartium ribicola, Urediniospores	5.5	100
Gymnosporangium clavipes, Aeciospores	4.0	100
Puccinia Malvacearum, Teliospores	2.0 3.0	Producing basidia 100. Producing basidiospores 0 Producing basidiospores 100
Sclerotinia fructigena, Conidia	5.0	100
Venturia inaequalis, Conidia	16.0 27.0	25 100

near the periphery of the cover glass germinate better than those near the center due to the relative amounts of air available. Duggar (**3**) found that a reduced oxygen supply retards spore germination. Blackman (**48**) noticed that if the germ tube of *Phragmidium violaceum* does not grow through the water and so reach the air it develops abnormally. Weimer (**9**) observed that when the teliospores of *Gymnosporangium Juniperi-virginianae* are covered with water they produce only long tubes instead of the normal promycelia and basidiospores. Melhus

and Durrell (7) found that when the urediniospores of *Puccinia coronata* are submerged only a small percentage of them germinate as compared with the number germinating when they float on a drop of water.

When spore germination tests were made in non-aerated distilled water, the writer secured best results by sowing the spores on the surface of the water rather than immersing them. Some spores, however, will not float. According to Duggar (3) the spores of many of the Phycomycetes and Hymenomycetes usually sink. The specific gravity of the spores of several Hymenomycetes determined by Buller (49) were between 1.02 and 1.21. Since some spores sink, all distilled water for germination studies should be aerated.

At this point reference should be made to the injurious effect of the presence of fungous spores on the germination of the spores of other fungi. Although the injurious effect of this competition between spores might be attributed to the secretion of toxic substances, there is little likelihood that this is the case. There is an indication that it is due to there being insufficient oxygen for all. The effect is much more marked in non-aerated than in aerated distilled water and is more marked in the interior of a drop of non-aerated distilled water than on its surface. All spores of Uredinales which the writer has observed germinate best when the drop of water in which they are contained is not contaminated by the presence of other fungous spores. When spores of *Alternaria*, *Colletotrichum*, or *Cladosporium* are present in a drop of water with the teliospores of *Puccinia Malvacearum*, the spores of the first three fungi may germinate but the teliospores of *Puccinia Malvacearum* do not germinate, although check teliospores in uncontaminated drops germinate perfectly. When in place of *Puccinia Malvacearum* the conidia of *Venturia inaequalis* were used a similar result was obtained; they germinated far better when no other spores were present with them in the drop of water. When there is lack of sufficient oxygen for all, only those spores which require the least will germinate. It was also observed that when relatively few spores of one species were present in a drop they germinated better than when the drop was crowded. This, too, may be attributed to insufficient oxygen. In all spore germination studies where optimum conditions are desired, an effort should be made to exclude from the culture drop the spores of all fungi except the

one under consideration, and there should not be present an excessive number of spores of that one fungus.

There are a few references which have come to the attention of the writer on the deleterious effect of competition or crowding on germinating spores. Edgerton (**50**) noticed that when more than twelve or fifteen spores of *Colletotrichum Lindemuthianum* are present in one cubic millimeter of water, they germinate more poorly than when a smaller number of spores are present. Taubenhaus (**51**) found it advisable to wash hollyhock leaves in water to remove the spores of saprophytic fungi preparatory to studying the germination of the teliospores of *Puccinia Malvacearum*. He does not indicate in what manner the presence of the spores of other fungi interfere with the germination tests.

Light relation.—Opinion in the literature is not unanimous as to the effect of light on spore germination. According to DeBary (**52**) and Farlow (**53**) light inhibits the germination of the spores of the Oomycetes. Cuboni (**54**) concluded that intense light interferes with the germination of the conidia of *Plasmopara viticola*. Ward (**55**) found that the spores of the brome rust germinate as readily in light as in darkness. Melhus (**22**) observed no difference in the percentage of conidia of *Cystopus canididus* germinating, nor in the time required for their germination, whether germination took place in light or darkness. Melhus (**23**) found that light does not interfere with the germination of the conidia of *Phytophthora infestans* if the optimum temperature for their germination is not exceeded. Duff (**17**) found that exposure to glass filtered sunlight is not injurious to the urediniospores of *Cronartium ribicola*, providing the temperature does not rise too high. He found that exposure to the ultra-violet rays from an electric arc completely inhibited spore germination. Lauritzen (**56**) concluded from his investigations that light is not a limited factor in the infection of plants by fungi.

The writer tested the effect of sunlight on the germination of the spores of *Alternaria Solani* and the conidia of *Sclerotinia fructigena*. The spores of both of these fungi germinate quite as well in sunlight, whether it be direct, diffuse, glass filtered or not glass filtered, as they do in darkness, provided that the conditions of temperature and moisture meanwhile remain near the optimum. The indications are that the spores of fungi

germinate in either light or darkness, when the other environmental conditions are between maximum and minimum limits. It is, however, probable that the germination of fungous spores in nature usually takes place in darkness, since it is in the night that conditions of temperatures and moisture nearer the optimum for germination usually prevail for a longer time.

Water relation.—An examination of the literature indicates that the moisture requirement of germinating spores is not uniform. Tulasne (**57**) was able to germinate teliospores of the Uredinales as well in a saturated atmosphere as in a drop of water. Patrigeon (**20**) found that for the germination of the conidia of *Plasmopara viticola* precipitated moisture is necessary and merely damp air is insufficient. According to DeBary (**47**) the spores of the Uredinales germinate better when they are merely in a moist atmosphere than when they are in water. Lesage (**58**) found that the spores of *Penicillium glaucum* can germinate in damp air so long as the humidity does not fall below 82 or 84 per cent. Taubenhaus (**51**) readily germinated the teliospores of *Puccinia Malvacearum* in a humid atmosphere. Levin (**59**) was able to infect tomatoes with *Septoria Lycopersici* by merely applying dry spores. Weimer (**9**) found the teliospores of *Gymnosporangium Juniperi-virginianae* unable to germinate when they were in contact with no moisture except that in the atmosphere. These spores did not germinate until the air became super-saturated, so that small drops of water collected on the slides in contact with the spores. Anderson (**11**) states that the spores of *Cylindrocladium scoparium* never germinate except when they are directly in water, a moist atmosphere being insufficient. According to Melhus and Durrell (**7**) the urediniospores of *Puccinia coronata* germinate only when they are in direct contact with water. Smiley (**33**) found that in the absence of a film of water, the spores of *Phyllosticta Antirrhini* do not germinate. Melhus, Durrell and Kirby (**18**) found that the sporidia of *Puccinia graminis* germinate profusely in drops of water, and but very poorly when their only moisture is obtained from the atmosphere.

The writer tested the effect of moist air as compared with precipitated moisture on the germination of the spores named in TABLE V. The aeciospores of *Gymnosporangium clavipes* germinate perfectly in moist air, as they do in water. The spores of *Alternaria Solani* and the conidia of *Venturia inaequalis* germinate

only 4 per cent in moist air as compared with 100 per cent in water. This means that in nature the relative number of spores of these two last named fungi germinating in moist air is very small, but undoubtedly sufficient to cause some infection. The conidia of *Sclerotinia fructigena* will not germinate in moist air but require precipitated moisture. The conidia of *Peronospora pygmaea* were also found to require precipitated moisture rather than moist air for their germination.

Of the spores studied by the writer and mentioned as above in the literature, in only five cases can they germinate well when their only source of moisture is the atmosphere. Three germinate very poorly in moist air. Six germinated only in precipitated moisture. The evidence is insufficient to warrant the conclusion that any group of fungi characteristically obtain their water for germination principally in the form of vapor. When optimum conditions for laboratory experiments on spore germination are to be obtained, water in the form of precipitated moisture rather than water vapor should be supplied. The question of too much water is probably closely related with the question of too little oxygen.

TABLE V

Effect of moist air as compared with precipitated moisture on the germination of fungous spores

Moisture conditions	Spore germinating (relative numbers)				
	A. Solani	*V. inaequalis*	*G. clavipes*	*S. fructigena*	*P. pygmaea*
In a drop of distilled water	100	100	100	100	100
On dry slide in moist chamber	4	4	100	0	0

SUMMARY

1. The germination of the following spores was studied; conidia of *Venturia inaequalis* (Cke.) Wint., conidia of *Sclerotinia fructigena* (Pers.) Schrt., spores of *Alternaria Solani* (E. & M.) Jones & Grout, spores of *Botrytis cinerea* Pers., spores of *Rhizopus nigricans* F. de Wal., aeciospores of *Gymnosporangium clavipes* C. & P., and teliospores of *Puccinia Malvacearum* Mont.

2. Spores of parasitic fungi germinate better when obtained from the living host than when obtained from artificial media.

3. Mature spores can germinate through a broader range of environmental conditions than can immature spores.

4. Freshly mature spores can germinate through a broader range of environmental conditions than can old spores. As the spores age, viability at first decreases sharply after which it is only gradually lost. Longevity of spores is dependent on conditions of storage after detachment from the host. Moisture is of more importance than temperature in its effect on the length of life of the spore.

5. Spores of the Phycomycetes can germinate at the lowest minimum temperatures, followed in order by the urediniospores, the aeciospores, and the teliospores of the Uredinales. Aeciospores have the lowest optimum temperatures for germination followed in order by urediniospores, conidia of Phycomycetes, teliospores, and the spores of the Imperfect Fungi.

6. The nearer all conditions are to the optimum, the shorter the time required for spore germination.

7. Competition or crowding inhibits spore germination. This is attributed to insufficient oxygen.

8. Spores of the fungi studied germinated in either light or darkness.

9. Precipitated moisture is unnecessary for the germination of some spores if water vapor is available.

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